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Kyburz, Andreas ; Müller, Anne

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The Gastrointestinal Tract Microbiota and Allergic Diseases

Andreas Kyburz Anne Müller

Institute of Molecular Cancer Research, University of Zurich, Winterthurerstr, Zurich, Switzerland

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Abstract
The gastrointestinal (GI) tract microbiota is required for optimal digestion of foods, for the development of resistance against pathogens (termed colonization resistance), for the development of mucosa-associated lymphoid tissue, and for local as well as systemic immune homeostasis. Certain constituents of the GI tract microbiota are widely recognized as critical regulators and modulators of their host's immune response. These include bacterial members of the microbiota as well as parasitic nematodes. Immune regulation by immunomodulatory members of the GI microbiota primarily serves to subvert host antimicrobial immune defenses and promote persistent colonization, but as a side effect may prevent or suppress immunological disorders resulting from inappropriate responses to harmless antigens, such as allergy, colitis or autoimmunity. Many of the best understood GI-resident immunomodulatory species have co-evolved with their mammalian hosts for tens of thousands of years and masterfully manipulate host immune responses. In this review, we discuss the epidemiological evidence for the role of the GI tract microbiota as a whole, and of specific members, in protection against allergic and other immunological disorders. We then focus on the mechanistic basis of microbial immunomodulation, which is presented using several well-understood paradigmatic examples, that is, helminths, *Helicobacter pylori*, Bifidobacteria and Lactobacilli. In a final chapter, we highlight past and ongoing attempts at harnessing the immunomodulatory properties of GI microbiota species and their secreted products for intervention studies and describe the promises and limitations of these experimental approaches. The effects of pro- and prebiotics, bacterial lysates, as well as of fecal microbiota transplantation are presented and compared.

Observational Studies Support a Critical Role of the GI Microbiota in Modifying Allergy Risk

Individual allergy risk is known to be influenced by environmental and lifestyle factors including diet, country of birth, exposure to antibiotics – especially early in life – sanitation, exposure to pets and livestock, the delivery mode, and breastfeeding, as well as genetic and epigenetic factors. Epidemiological and experimental studies support the hypothesis that the composition and diversity of the gastrointestinal (GI) microbiota heavily influences the risk of the development of allergic diseases.

Modern culture-independent techniques have provided a comprehensive picture of the gut microbiota, its di-

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Anne Müller
Institute of Molecular Cancer Research
University of Zurich
Winterthurerstr. 190, CH–8057 Zurich (Switzerland)
E-Mail mueller@imcr.uzh.ch

versity and alterations due to environmental influences [1]. They are based on the amplification and sequencing of parts of the 16S rRNA gene [1, 2]; alternatively, metagenome or metatranscriptome sequencing is applied to obtain a higher resolution overview of the microbiota and to allow the detection of archaea, fungi, and viruses in addition to bacteria [1–3]. Other emerging methods are metaproteomic and metabolomic analyses, aiming to characterize the functionality of the microbiome [2, 3]. Through application of these modern culture-independent as well as culture-dependent methods, it has become increasingly clear that the composition of the gut microbiota is strongly influenced by environmental and lifestyle factors, which in turn affect the risk of allergic and other non-communicable diseases (NCDs). Better personal hygiene, smaller family size, dietary changes, and the excessive use of antibiotics in industrialized countries have all been held responsible for changes in the gut microbiota and allergy risk [4–6]. Several studies have reported beneficial effects on allergic outcomes of growing up in rural farming environments; livestock exposure, and therefore, early or prenatal microbial exposure appears to account for the lower allergy risk of farmers' children [7, 8], which has been attributed mechanistically to differences in innate immune responses, as well as increased number and functions of cord blood regulatory T cells [9, 10]. Moreover, bacterial communities in dust samples isolated from households with dogs or cats were found to be richer and more diverse, and exposure to such environments during infancy is known to protect against allergic disease development in childhood [11, 12].

A reduced GI tract microbiota diversity is clearly linked to early-onset NCDs, including atopy [13], eczema [14–17], and asthma [18]. The reduced GI microbiota diversity in allergic children is dominated by Firmicutes [19–21], and members of the *Bacteroidaceae* family [22] and more specifically, by increased numbers of *Bacteroides fragilis* [23, 24], *Escherichia coli* [25], *Clostridium difficile* [17, 25, 26], *Bifidobacterium catenulatum* [19–21, 27], and *Bifidobacterium longum* [28–30], and a lower prevalence of *Bifidobacterium adolescentis* [28–30], *Bifidobacterium bifidum* [28–30], and *Lactobacillus* species [29–31]. These general trends are not confirmed by all studies; for instance, a study in Norway found a lower rather than higher concentration of *E. coli* among allergic individuals [28]. Also, in a study by Verhulst et al. [32], children who developed wheezing had a lower prevalence of *C. difficile*. The reduced exposure of Western populations to microbes that are not classically categorized as constituents of the commensal microbiota, such as the gastric colonizer and pathobiont *Helicobacter pylori* [33–35], or intestinal helminths [36, 37], also increases the risk of allergic diseases.

Lifestyle factors such as delivery mode and breast-feeding strongly affect the establishment of the human gut microbiota and the risk of allergic outcomes. The recent finding that bacterial DNA is present in the newborn's first stool and in the fetoplacental unit suggests that the acquisition of the intestinal microbiota may already begin in utero and is then further shaped during birth and postnatally [38–40]. At birth, first major microbial exposures originate from the maternal vaginal and perianal microbiota. Consequently, the intestinal microbiota of newborns resembles that of his or her mother's vagina [41]. In contrast, neonates delivered by means of cesarean section (CS) acquire a gut microbiota that is similar to the one found on maternal skin. This is then followed by the typically slower (compared to vaginally delivered infants) acquisition of a more complex microbiota [42, 43]. Infants born by means of CS are at higher risk for respiratory distress [44], asthma and atopy [45], as well as obesity [46], and type I diabetes [47]. Furthermore, CS involves antibiotic exposure and can delay the onset of breastfeeding, which negatively influence the establishment of a normal healthy gut microbiota [14, 48, 49]. Indeed, breastfed (>4 months) neonates exhibited a reduced risk of developing asthma until 8 years of age [50]. Evidence is available that breastfeeding protects against atopic dermatitis, wheeze in early childhood and cow's milk allergy [51]. However, the data are inconsistent, as other studies have reported no benefit of breastfeeding in children from non-atopic families or in decreasing the risk of asthma in infants at 5 years of age [52, 53]. Overall, the aforementioned studies imply that early-life or even prenatal exposure to microbes is critical for shaping a healthy GI tract microbiota, which can lower allergy risk later in life.

Mechanisms of Microbially Induced Immune Tolerance and Protection Against Allergic Disorders

Intestinal Helminths Protect against Allergy, IBD and Autoimmunity by Suppressing Innate Lymphoid Cell Activation and Promoting Treg Differentiation

Epidemiological and experimental studies have documented a clear inverse association between helminth infections and allergic, chronic inflammatory and autoimmune disease manifestations (reviewed in [54, 55]). Parasites release immunomodulatory products that have evolved to suppress Th2-driven immunity and to ensure

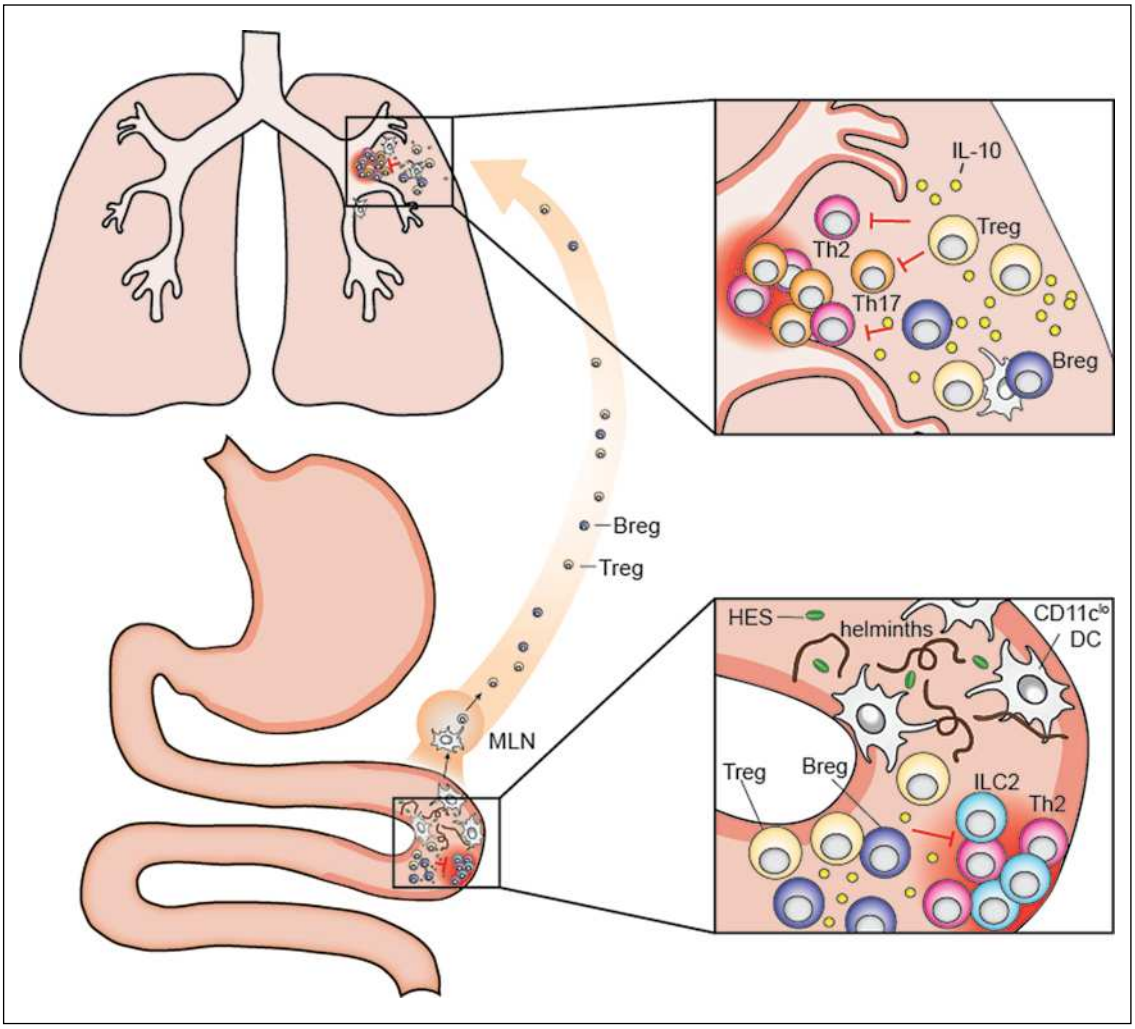

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Fig. 1. Allergy-preventive mechanisms of intestinal helminths. Helminths and other parasitic worms colonize the small intestine of humans in tropical and subtropical geographic regions of the world. The nematode species *H. polygyrus* is used most commonly for experimental infections of various susceptible mouse strains. Helminths and their HES products locally suppress the activation of and cytokine production by type 2 ILCs, and target CD11c^{lo} DC subsets to promote their tolerogenic activity. Tregs and Bregs have been implicated in suppressing allergen-specific immune responses in the lungs through both IL-10-dependent and -independent processes.

helminth persistence, and that, when administered to uninfected mice, reproduce many of the beneficial effects of live infection [56]. One of the most commonly used murine models of helminth infection takes advantage of the mouse intestinal nematode species *Heligmosomoides polygyrus*, which can be administered alive in larval form and which will colonize virtually all laboratory mouse strains, and of its excretory/secretory products (*H. polygyrus* excretory/secretory antigen, helminth excretory/secretory (HES)). Experimental *H. polygyrus* infection triggers a Th2-predominant immune response that is accompanied by regulatory T-cell expansion and activation in the draining mesenteric lymph nodes (MLN) [57] and that typically fails to eliminate the parasites. Live nematode parasite infection of mice, as well as the adoptive transfer of MLN-derived Tregs from infected donors to uninfected recipients, suppresses allergen-induced airway hyper-responsiveness in the recipients and confers protection against allergy [58] (fig. 1). The treatment of naive T-cells with HES in conjunction with a TCR ligand was shown to trigger the expression of the Treg lineage-defining transcription factor FoxP3 in vitro through the stimulation of TGF-β receptor II signaling, generating Tregs with suppressive activity in asthma models [59]. A

more recent report on Treg depletion in asthma models has, however, challenged the critical role of FoxP3$^+$ Tregs in HES-induced asthma protection, instead implicating effects on innate lymphoid cells (ILCs) in allergen-specific immune suppression. This study found that the alum-adjuvanted sensitization with ovalbumin, which constitutes the first phase of allergen-induced asthma in the widely used alum/ovalbumin-induced model of the disease, triggers the production of IL-5, IL-13 and other cytokines from type 2 ILCs, which is strongly suppressed by HES [60] (fig. 1). Similar findings have been reported in an adjuvant-independent model of fungal extract-induced airway hyper-responsiveness [56]. In addition to Tregs and ILCs, dendritic cells (DCs) have been implicated in immunosuppression by both live parasites and HES. Treatment with HES inhibits DC-specific cytokine production upon TLR activation, as well as other DC functions [61], a phenomenon that may be recapitulated in vivo by the expansion and tolerogenic Treg-inducing activity of CD11c^{lo} DCs in the context of *H. polygyrus* infection [62]. In summary, many of the critical cellular and molecular players driving helminth-specific immunomodulation have been identified in the *H. polygyrus* mouse model; in contrast, comparatively little is known about the correlates of allergy protection by parasites in humans. Helminth-infected humans are known for their general T-cell hyporesponsiveness [63], which in experimental studies on helminth (Ascaris, Trichuris)-infected children in Indonesia has been attributed to the higher suppressive activity of their FoxP3$^+$ Tregs relative to those of uninfected children [64]. Other studies have documented higher frequencies of FoxP3$^+$ Tregs in *Schistosoma*-infected children relative to their uninfected counterparts [65]. An additional, less well understood subset of regulatory lymphocytes, that is, regulatory B-cells (Bregs), has also been implicated in helminth-induced allergy protection in humans [66]. Adoptive transfer experiments have confirmed the protective activity of Bregs in models of allergen-induced asthma [66]. The CD24^{hi} CD27$^+$ subset of B-cells, through its capacity to secrete IL-10, appears to be specifically involved in helminth-specific immuno-suppression; both the frequency and functionality of this subset are reduced in allergic individuals [67].

The Gastric Pathobiont H. pylori Suppresses Allergic Asthma and IBD by Promoting Tolerogenic DC and Regulatory T-Cell Responses

H. pylori is an ancestral constituent of the gastric microbiota that has colonized and co-evolved with its human host for at least 60,000 years [68]. *H. pylori* causes histologically evident, but mostly asymptomatic gastritis in all carriers, which may progress to more severe gastric disorders such as gastric and duodenal ulcers, and gastric cancer or B-cell lymphoma (recently reviewed in [69]). Whether an infected individual will develop a clinically overt disease depends on the host genetic predisposition, virulence of the infecting strain, and the polarization and strength of the host immune response to the infection [70]. While T-cell responses of patients with *H. pylori*-associated diseases, such as duodenal ulcer, are generally Th1/Th2-driven, T-cell responses in asymptomatic carriers tend to be Treg-predominant, with high levels of IL-10 and TGF-β production [71]. Similarly, children who rarely suffer from *H. pylori*-associated diseases despite being colonized at high levels, predominantly mount Treg responses to their strain [72]. Despite its strict host specificity (*H. pylori* has not been isolated from any other mammalian species), certain isolates of *H. pylori* can be used for experimental infection of mice [73, 74]. The requirements for eliciting histologically evident gastric disease – atrophic gastritis, intestinal metaplasia and other preneoplastic lesions – are similar in the human and murine host, with a specific contribution of the Cag pathogenicity island to premalignant transformation being observed in both settings [73, 75]. Experimental murine infection can be used to recapitulate both asymptomatic carriage and *H. pylori*-associated disease, with the outcome being driven by a single variable, that is, the age at the time of first exposure [73]. While mice infected during adulthood develop chronic gastritis and preneoplastic lesions over time, mice infected during the neonatal period will mimic the asymptomatic human carrier and be protected against gastric pathology [73] (fig. 2). The neonatal infection model generates immune tolerance towards *H. pylori* that is driven by highly suppressive, CD4$^+$CD25^{hi}FoxP3$^+$ Tregs, which suppress anti-*H. pylori* T-effector cell-mediated immunity [73]. An interesting side effect of neonatally induced immune tolerance is the efficient protection of infected mice against allergic asthma, which is evident in ovalbumin/alum- and house dust mite-induced asthma models [76, 77]. Experimental data reflect epidemiological studies in human populations, which have documented an inverse association of *H. pylori* infection with the risk of developing allergic asthma, atopic rhinitis and other allergic disease manifestations [78–82]. The inverse association of *H. pylori* status and allergy risk was particularly striking in children and early-onset asthmatics, suggesting that young individuals benefit most from harboring *H. pylori* [78, 80, 81]. As in hel-

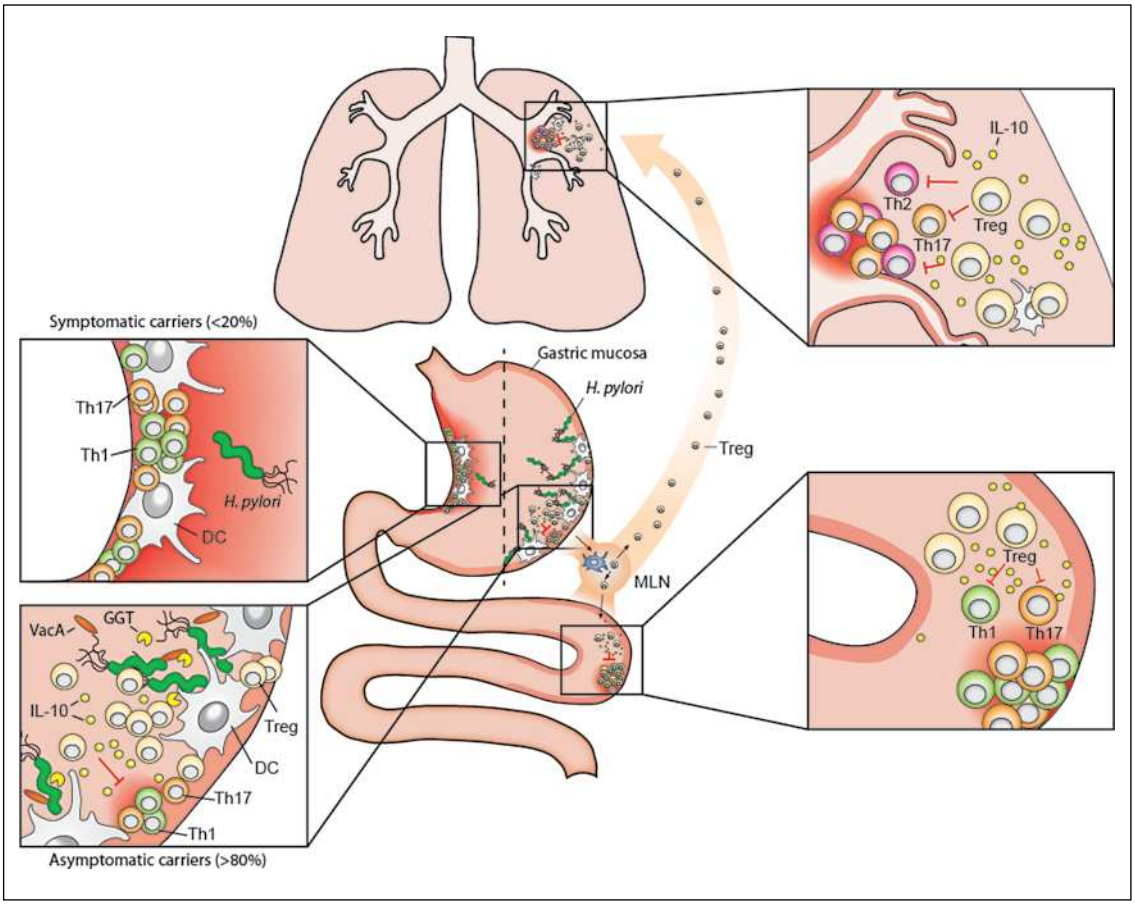

Fig. 2. The gastric pathobiont *H. pylori* is typically acquired during early childhood, and is known to cause gastric disorders, as well as prevent allergic and chronic inflammatory diseases. *H. pylori* colonizes the gastric mucosa of roughly one half of the human population. Ten to 20% of infected individuals will develop clinically overt disease (such as chronic gastritis and gastric ulcers, shown in the upper left inset) that is driven by pathogenic T-cells. Greater than 80% of the infected population will remain asymptomatic throughout life despite harboring high levels of *H. pylori* (lower left inset). Both outcomes can be recapitulated in experimentally infected mice. The *H. pylori* persistence factors GGT and VacA promote chronic infection by tolerizing DCs and thereby promoting Treg differentiation. *H. pylori*-induced Tregs are required for the suppression of allergen-specific immune responses in the lung and for the alleviation of colitis symptoms in models of inflammatory bowel disease (upper and lower right insets). Treg- and DC-derived IL-10 contributes to *H. pylori*-specific immunomodulation.

minth infections, Tregs appear to be the main cellular players driving tolerance to allergens (fig. 2); the depletion of Tregs breaks tolerance and abrogates protection, whereas the adoptive transfer of MLN-derived $CD25^+$ Tregs is sufficient to protect naive recipients against allergic asthma [73, 76]. Several *H. pylori* factors are critical for Treg differentiation in the context of neonatal infection with the bacteria; in particular, the secreted factors γ-glutamyl-transpeptidase (GGT) and the vacuolating cytotoxin (VacA) both non-redundantly contribute to Treg differentiation, immune tolerance and asthma protection [83] (fig. 2). Both are essential persistence factors in *H. pylori* infection [83] and expressed in all strains; both factors can further be administered in purified form to recapitulate all critical features of immune tolerance and allergy protection observed with live infection [84]. Treg differentiation upon exposure to $VacA^+GGT^+$ *H. pylori* requires DCs, which are actively targeted and 'tolerized' by *H. pylori* via the two secreted factors [83]. *H. pylori*-experienced DCs retain a semi-mature phenotype, expressing high levels of MHCII, but low or no costimulatory molecules [83, 85]. Semi-mature DCs are tolerogenic, that is, they exhibit a high propensity for priming Treg differentiation, but have only a limited ability to prime effector T-cell responses [86, 87]. The tolerogenic DC/Treg axis likely accounts for the protection of

H. pylori-infected individuals not only against allergic diseases but also against IBD [88–91] (fig. 2) and potentially against auto-immunity, as demonstrated in models of multiple sclerosis [92]. Overall, much can be learned about general microbial immune modulation by studying *H. pylori* due to readily available experimental infection models and the ease of genetic manipulation of these highly persistent colonizers of the human stomach.

Immunomodulatory Lactobacilli and Bifidobacteria Induce IL-10 and Retinoic Acid Expression in DCs and Promote Treg Differentiation

Given the large amount of interventional trials using various strains of Lactobacilli and Bifidobacteria (see chapter below), it is astonishing how little experimental information is available regarding the mechanistic basis underlying the protective effects of these classes of microbes on allergic and other immunologically driven diseases. However, a few concepts have emerged that apply to more than one strain and have been tested in rigorous experimental systems. One such model system utilizes *Bifidobacterium infantis*, a strain that was originally isolated from surgically removed, human intestinal mucosa and was shown to adhere closely to epithelial cells without inducing NF-κB activation and secretion of cytokines and chemokines [93]. Moreover, *B. infantis* co-culture not only fails to induce, but actively suppresses the release of pro-inflammatory molecules by epithelial cells exposed to live *Salmonella* spp. or purified TLR ligands; similar effects are observed with another immunomodulatory organism, *Lactobacillus salivarius* [93, 94]. A second, non-epithelial target cell of *B. infantis* is the antigen-presenting cell, especially the DC. Upon sampling antigenic material at sites of pathogen entry, such as the GI mucosa, immature sentinel DCs undergo maturation and migrate to the tissue-draining lymphoid organs, where they present pathogen-specific and co-stimulatory molecules (MHC peptide complexes and B7 family molecules) to naive T-helper cells. A third, soluble signal provided by the DC to the naive T-cells during antigen presentation determines the polarization of the T-cell into Th1, Th2, Th17 or Treg lineages; the nature of the third signal is governed by the conditions encountered by the DCs during antigen sampling. Interestingly, DCs directly cultured or derived from various human sources (MLN, blood) all consistently secrete IL-10, TGF-β and retinoic acid, but not IL-12 or TNF-α, upon co-culture with *B. infantis* [95, 96]. IL-10, TGF-β and retinoic acid all contribute to the differentiation of naive T-cells into Tregs rather than Th1 or other T-effector cells, a well-documented consequence of *B. infantis* administration to mice [96]. The $CD103^+$ DC subset in the intestinal lamina propria is especially equipped to produce retinoic acid and promote Treg differentiation; high numbers of intestinal Tregs in turn drive the protection against colitis [96] and allergy that is a hallmark of Bifidobacteria (*B. infantis* and other strains) feeding to mice [97]. Administration of *B. infantis* to human volunteers induced increased frequencies of $FoxP3^+$ Tregs in peripheral blood [95], lending further support to the notion that probiotic *Bifidobacterium* and *Lactobacillus* species alleviate allergic and chronic inflammatory diseases via the DC/IL-10/retinoic acid/Treg axis.

Interventional Trials Confirm Beneficial Effects of Probiotics on Allergic Disease Risk and Severity

In the past decades, a range of microbiota-derived products has been tested in human clinical trials of allergic and other immunological diseases. The applications can be divided into prebiotics, probiotics, bacterial lysates (BLs) and fecal microbiota transplantation (FMT). A majority of intervention studies have examined probiotic bacteria. Most studies monitor early outcomes of allergic diseases such as eczema and IgE-mediated food allergy, whereas only a few studies examine longer-term outcomes such as respiratory allergic disease.

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host [98]. The first medical application of live bacteria was *E. coli* Nissle 1917, which was isolated from a soldier who survived an epidemic of diarrhea during World War 1 [99]. The potential of live microorganisms was thereafter neglected for a long time in favor of pharmaceutical interventions. Most recent studies have been conducted using strains of Lactobacilli and Bifidobacteria, and they tend to show benefits in allergy prevention but not in the treatment of established allergies [38]. Several very recent trials published in 2013–2015, however, have raised new hopes that a reduction of symptoms in adult and pediatric allergic rhinitis, eczema and food allergy can be achieved by the use of probiotics (table 1). Systematic reviews and meta-analyses suggest that combined pre- and postnatal probiotic supplementation is most efficacious in preventing the development of infant eczema and atopic eczema [100–103], but no convincing data on beneficial effects on later-onset allergic diseases such as wheezing, allergic asthma or other allergic outcomes have been reported to date [104–107]. The lack of

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Table 1. Recent clinical trials using probiotics, prebiotics or bacterial lysates to treat or prevent allergic diseases

Intervention	Compound/strain	Indication	Time point of treatment	Title	Description/groups	Main result	Author/year
Probiotics	*Lactobacillus paracasei* (LP) and *Lactobacillus fermentum* (LF)	Atopic dermatitis	Postnatal	Children with atopic dermatitis show clinical improvement after *Lactobacillus* exposure	220 children aged 1–18 years with moderate-to-severe atopic dermatitis (AD). Randomized to receive LP, LF, LP + LF mixture, and placebo for 3 months	Lower severity 4 months after discontinuation of treatment and higher quality of life for LP, LF, LP + LF treatment compared to placebo	Wang et al., 2015
	Bifidobacterium bifidum	Eczema	Postnatal	Protective effect of probiotics in the treatment of infantile eczema	40 infants with eczema	After 4 weeks of treatment with *B. bifidum*, the levels of B. bifidum increased sharply and the severity index was notably reduced	Lin et al., 2015
	Lactobacillus rhamnosus HN001 (HN001) and *Bifidobacterium animalis* subsp. lactis HN019 (HN019)	Eczema	Postnatal	Differential effects of 2 probiotics on the risks of eczema and atopy associated with single nucleotide polymorphisms (SNPs) in toll-like receptors	331 high-risk infants with a predisposition to eczema conferred by genetic variation in toll-like receptor genes (54 SNPs), receiving either placebo, HN001 or HN019	Negative impact of specific TLR genotypes may be positively affected by probiotic supplementation. HN001 exhibits a much stronger effect than HN019 in this respect	Marlow et al., 2015
	Lactobacillus rhamnosus CGMCC 1.3724	Food allergy	Postnatal	Administration of a probiotic with peanut oral immunotherapy (PPOIT): a randomized trial	62 children (1–10 years) with peanut allergy	Induction of sustained unresponsiveness 2–5 weeks after discontinuation of treatment. Reduced peanut skin prick test responses and peanut-specific IgE levels	Tang et al., 2015
	Lactobacilluss casei, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Bifidobacterium lactis*	Atopic dermatitis	Postnatal	Efficacy of probiotic therapy on atopic dermatitis in children: a randomized, double-blind, placebo-controlled trial	100 children with mild to moderate AD (2–9 years old) randomly assigned to the probiotics mixture (*Lactobacilluss casei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Bifidobacterium lactis*) or placebo groups	No therapeutic or immunomodulatory effects on the treatment of AD after 6 weeks	Yang et al., 2014
	Lactobacillus rhamnosus HN001 (HN001) and *Bifidobacterium animalis* subsp. lactis HN019 (HN019)	Eczema	Postnatal	Differential modification of genetic susceptibility to childhood eczema by 2 probiotics	331 high-risk infants with a predisposition to eczema conferred by 33 SNPs, receiving either placebo, HN001 or HN019	Children at a high risk of developing eczema were less likely to develop eczema if they had been randomized to the HN001 group compared to those in the placebo group. HN019 was also able to protect against the effects of some SNPs	Morgan et al., 2014
	Bifidobacterium animalis subsp. lactis LKM512	Atopic dermatitis	Adult	Antipruritic effects of the probiotic strain LKM512 in adults with atopic dermatitis	44 adult AD patients were randomly assigned to receive LKM512 or a placebo and underwent medical examinations	Alleviated itch in AD patients and improved the dermatology-specific quality-of-life scores and reduced pruritus compared to controls	Matsumoto et al., 2014

Table 1. (continued)

Intervention	Compound/strain	Indication	Time point of treatment	Title	Description/groups	Main result	Author/year
	Lactobacillus paracasei subsp. paracasei LP-33	Allergic rhinitis	Adult	Efficacy and safety of the probiotic *Lactobacillus paracasei* LP-33 in allergic rhinitis: a double-blind, randomized, placebo-controlled trial	425 patients with allergic rhinitis (AR) to grass pollen treated with loratadine and presenting altered quality of life	LP-33 improves the quality of life of subjects with persistent AR who are currently being treated with an oral H 1 -antihistamine. Whereas nasal symptoms had not changed, ocular symptoms had consistently improved	Costa et al., 2014
	Lactobacillus salivarius CUL61, *Lactobacillus paracasei* CUL08, *Bifidobacterium animalis* subspecies lactis CUL34 and *B. bifidum* CUL20	Eczema	Pre- and postnatal	Probiotics in the prevention of eczema: a randomised controlled trial	Pregnant women from 36 weeks gestation and their infants to age 6 months received either the probiotic or placebo daily	No evidence that the probiotic either prevented eczema during the study (up to 2 years) or reduced its severity. However, the probiotic seemed to prevent atopic sensitisation to common food allergens and to thereby reduce the incidence of atopic eczema in early childhood	Morgan, 2014
	Lactobacillus paracasei (LP), strain HF.A00232	Allergic rhinitis	Postnatal	Evaluation of the effect of *Lactobacillus paracasei* (HF. A00232) in children (6–13 years old) with perennial allergic rhinitis: a 12-week, double-blind, randomized, placebo-controlled study	60 children with AR aged 6–13 years with nasal total symptoms score (NTSS) 5. Two groups with 28 participants receiving levocetirizine plus placebo and 32 participants receiving regular levocetirizine plus LP (HF.A00232) for the first 8 weeks, with a shift to levocetirizine as rescue treatment during the following 4 weeks	No additional benefit when used with regular levocetirizine in treating AR in the initial 8 weeks of study, but there was a continuing increase in the quality of life, as well as a significant improvement in individual symptoms	Lin et al., 2014
	Lactobacillus paracasei NCC2461	Allergic rhinitis	Adult	Comparison of 2 oral probiotic preparations in a randomized crossover trial highlights a potentially beneficial effect of *Lactobacillus paracasei* NCC2461 in patients with allergic rhinitis	31 adults with allergic rhinitis to grass pollen	Despite short-term consumption, NCC2461 was able to reduce subjective nasal pruritus while not affecting nasal congestion in adults suffering from grass pollen allergic rhinitis	Perrin et al., 2014
	Lactobacillus paracasei ssp. paracasei F19 (LF-19)	Eczema, allergic rhinitis, asthma, and food allergy	Postnatal	Probiotics in primary prevention of allergic disease – follow-up at 8–9 years of age	171 infants were randomized to daily intake of cereals with (n = 89) or without LF19 10 (8) CFU (n = 90) from 4 to 13 months of age. At age 8–9 allergic disease manifestations were evaluated	No long-term effect of LF19 on any diagnosed allergic disease, airway inflammation or IgE sensitization. Delayed eczema onset, but larger study population needed	West et al., 2013
	Lactobacillus salivarius	Allergic rhinitis	Postnatal	Effect of probiotics on allergic rhinitis in Df, Dp or dust-sensitive children: a randomized double blind controlled trial	199 atopic children with current allergic rhinitis received probiotics or placebo daily for 12 weeks	Reduction in rhinitis symptoms and drug scores but no difference for any immunological or blood cell variables	Lin et al., 2013

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Intervention	Compound/strain	Indication	Time point of treatment	Title	Description/groups	Main result	Author/year
	Lactobacillus reuteri	Eczema	Pre- and postnatal	No effect of probiotics on respiratory allergies: a 7-year follow-up of a randomized controlled trial in infancy	Last month of gestation and through the first year of life comprising 232 families with allergic disease, of whom 184 completed a 7-year follow-up	Reduced the incidence of IgE-associated allergic disease in infancy, but no effects were observed later on the prevalence of respiratory allergic disease at school age	Abrahamsson et al., 2013
	(NCC)2818 *Bifidobacterium lactis*	Allergic rhinitis	Adult	Immune-modulatory effect of probiotic *Bifidobacterium lactis* NCC2818 in individuals suffering from seasonal allergic rhinitis to grass pollen: an exploratory, randomized, placebo-controlled clinical trial	Adults with seasonal allergic rhinitis received probiotics or placebo for 8 weeks	The probiotic NCC2818 mitigates immune parameters and allergic symptoms during seasonal exposure	Singh et al., 2013
	Lactobacillus GG (LGG)	Eczema, allergic rhinitis, and food allergy	Pre- and postnatal	Prenatal and postnatal probiotics reduces maternal but not childhood allergic diseases: a randomized, double-blind, placebo-controlled trial	191 pregnant women with atopic diseases determined by history, received daily from 24-week gestation until delivery probiotics or placebo. After delivery breastfeeding mothers were further treated and non-breastfeeding infants also for 6 months	Administration of LGG from 24 weeks' gestation reduced severity of maternal atopy but did not prevent childhood sensitization or allergic disease	Marks et al., 2013
Prebiotics	Fructooligosaccharides-inulin	Atopic dermatitis	Postnatal	Objective score of atopic dermatitis and prebiotic in infant	70 7–24 month old infants with AD received either prebiotics or placebo for 3 months	Prebiotics more strongly reduced clinical signs compared to placebo	Ghanei et al., 2014
	Neutral and acidic oligosaccharide	Atopic dermatitis	Postnatal	Effect of non-human neutral and acidic oligosaccharides on allergic and infectious diseases in preterm infants	113 preterm infants (gestational age <32 weeks and/or birth weight <1,500 g) were allocated to receive enteral neutral and acidic oligosaccharide supplementation or placebo between days 3 and 30 of life	Supplementation of non-human neutral and acidic oligosaccharides in preterm infants does not decrease the incidence of allergic diseases during the first year of life	Niele et al., 2013
	Oligosaccharides (scGOS/lcFOS)	Food allergy	Postnatal	Effect of the specific infant formula mixture of oligosaccharides on local immunity and development of allergic and infectious disease in young children: randomized study	80 infants who were breastfed, 80 infants consuming the formula supplemented with oligosaccharides, 80 infants fed with a standard formula	Infants fed with breast milk and supplemented formula had significantly less allergic reactions to food products up to 18 months of life compared to the babies from the third group	Ivakhnenko et al., 2013
Bacterial Lysates	Heat-killed Gram-negative Escherichia coli Symbio and Gram-positive Enterococcus faecalis Symbio	Atopic dermatitis	Postnatal	What did we learn from farm studies: state of the art clinical studies with bacterial lysates for allergy prevention	606 newborns with at least single heredity for atopy. Intervention phase from week 5 to the end of month 7 was followed by a monitoring phase until 3 years of age	Reduction of the prevalence of atopic dermatitis in the subgroup of infants with single heredity for atopy	Lau, 2013

efficacy in other allergic disorders may be attributable to the time point of treatment onset, which is often late in the last trimester of pregnancy or post-natally [108] (table 1). Initiating interventions as early as the beginning of the second trimester has been found to increase efficacy [109]. Unconventional probiotics like *Faecalibacterium prausnitzii*, *Akkermansia muciniphila*, certain *Clostridia* or *Bacteroides* species may prove to be more promising than the predominantly used strains of Lactobacilli and Bifidobacteria in preventing allergic diseases [110]. The use of helminths has been propagated to treat allergies and inflammatory diseases, given the promising results from preclinical trials. However, helminths did not confer beneficial effects in allergic asthma patients, and outcomes for other atopic disorders in humans have been inconsistent [111].

BLs, that is, extracts from defined bacterial cultures, have originally been developed to prevent recurrent acute respiratory tract infections (ARTIs), including the common cold [112]. ARTIs are reported to exacerbate asthma and wheezing in childhood and to increase the risk of allergies later in life [113]. The best understood BL, OM-85 BV (also known as Bronchovaxom) consists of extracts of a mixture of bacterial pathogens causing respiratory tract infections [112]. Human clinical studies have revealed beneficial effects of OM-85 BV on the recurrence of ARTIs and a reduction of associated symptoms [113–116]. Moreover, other BLs have been shown to reduce the recurrence and duration of ARTIs [117, 118] as well as a reduction of atopic dermatitis in infants at heredity risk for atopy [119] (table 1).

Motivated by promising experimental data in mice, several clinical trials have assessed the allergy-preventive effects of various non-digestible, fermentable oligosaccharides that are summarized under the term prebiotics. These diverse fibers promote the growth of endogenous gut bacterial species such as Bifidobacteria and Lactobacilli and thereby enhance microbial metabolic processes producing short chain fatty acids (SCFAs) with anti-inflammatory properties [120, 121]. A meta-analysis and systematic review of several studies found a significant reduction of eczema in infants and a reduction in asthma in infants at high risk of allergy upon prebiotic supplementation [122]. Recently, two trials could show decreased allergic food reactivity in young children after postnatal prebiotic application as well as alleviated clinical signs in infants with atopic dermatitis (see table 1 for most recent trials). However, due to the shortage of available data, the benefits of prebiotics on outcomes other than eczema remain to be examined [122, 123].

FMT has attracted increased attention lately due to its great efficacy in the treatment of *C. difficile*-induced diarrhea. Several FMT trials for the treatment of IBD have recently been evaluated in a systematic review and meta-analysis. Overall, the remission rate was significantly increased, mainly for Crohn's disease (60.5%) and younger patients (7–20 years, 64.1%) and less convincingly for ulcerative colitis with a remission rate of only 22% [124, 125]. Moreover, FMT is currently being investigated as a therapy for pediatric allergic disorders [125].

Conclusions and Future Perspectives

Observational, experimental and interventional studies all point to a critical role of a healthy and diverse GI tract microbiota in directing tolerance to harmless environmental or auto-antigens. Although experimental research and human trials have mostly focused on Lactobacilli and Bifidobacteria in the past, other, less well understood members of the microbiota may turn out to have stronger immunomodulatory effects and exhibit better efficacy in the prevention and treatment of allergic disorders. Among these are the gastric pathobiont *H. pylori* and intestinal helminths; both are persistent colonizers of their respective niche and share the ability to induce tolerogenic activity in DCs and Treg differentiation in naive T-cells. Longer interventional trials are needed to establish efficacy beyond atopic eczema in children, and to assess possible beneficial effects of pre- and postnatal treatment strategies on the allergy risk of adolescents and adults. The epigenetic basis for the intergenerational transmission of microbial immunomodulatory effects will receive increasing attention, and, in combination with global microbiome analyses at the genomic, transcriptomic and proteomic levels, will lead to a better understanding of the regulation of tolerance vs. allergy by the constituents of a healthy GI tract microbiota.

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